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INTEGRATIVE GC–MS AND MOLECULAR DOCKING ANALYSIS OF BIOACTIVE COMPOUNDS FROM *GRATELOUPIA LITHOPHILA* TARGETING GASTRIC CARCINOGENESIS

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Abstract:

The present study investigated the metabolomic profile and therapeutic potential of Grateloupia lithophila Borgesen (Halymeniaceae), a marine red alga with promising pharmacological applications. Ethanolic extracts of the alga were analyzed using Gas Chromatography–Mass Spectrometry (GC–MS), revealing a chemically diverse composition rich in fatty acids, terpenoids, steroids, esters, and polyphenolic compounds. A total of 68 metabolites were identified, with n-hexadecanoic acid (47.87%), oleic acid (12.61%), 7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid (5.96%), and octadecanoic acid (5.17%) as the major constituents. Several compounds, including ageratriol, supraene, dimethylaminoethyl palmitate, benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate, and a xanthene derivative, were reported for the first time in this species. Molecular docking studies against seven gastric cancer-related protein targets demonstrated strong binding affinities for cholesta-4,6-dien-3-ol (3β), methyl 7α-hydroxy-3α-methoxy-5β-cholanoate, and ageratriol. These compounds exhibited favorable molecular interactions, suggesting potential anticancer activity through kinase inhibition and apoptosis regulation. Additionally, sterol derivatives may possess antibacterial activity against Helicobacter pylori, supporting their relevance in gastric cancer prevention and therapy.

Keywords: *Grateloupia lithophila, GC-MS Analysis, Molecular Docking, Gastric Cancer, Marine Bioactive Compounds.*

Introduction

Marine algae have emerged as prolific reservoirs of structurally diverse bioactive compounds with significant therapeutic potential. Among them, red algae (Rhodophyta) represent a chemically rich group known for producing unique secondary metabolites, including sulfated polysaccharides, sterols, terpenoids, fatty acids, and phenolic derivatives, which exhibit a broad spectrum of pharmacological activities such as antioxidant,

antimicrobial, anti-inflammatory, and anticancer effects [1,2,3]. Within this group, the genus *Grateloupia* (family Halymeniaceae) has gained increasing scientific interest for its multifaceted biological properties and metabolomic diversity [4]. Several species, including *Grateloupia filicina*, *G. turuturu*, and *G. livida*, have been extensively studied for their potent cytotoxic, antitumor, and hepatoprotective activities, primarily attributed to sterols and terpenoid constituents [5,6,7].*Grateloupia lithophila* Borgesen, a lesser-explored red alga distributed along the Indian and Indo-Pacific coasts, has recently drawn attention due to its rich phytochemical composition and potential biomedical applications [8]. Preliminary reports suggest that *G. lithophila* contains bioactive compounds with antioxidant and antibacterial properties; however, its anticancer potential remains poorly elucidated. The escalating incidence of gastric carcinoma worldwide underscores the urgent need for natural and multifunctional therapeutic agents capable of targeting multiple oncogenic pathways with reduced side effects [9,10]. Moreover, the strong association between *Helicobacter pylori* infection and gastric cancer progression highlights the necessity of identifying dual-action molecules that can simultaneously inhibit bacterial proliferation and modulate tumor signaling mechanisms [11,12]. GC-MS analysis is a powerful technique widely used to identify and quantify phyto-constituents (bioactive compounds derived from plants). It combines the separation capabilities of Gas Chromatography (GC) with the detection power of Mass Spectrometry (MS). GC-MS analysis is essential in phyto-constituent studies for identifying and quantifying complex plant extracts with high sensitivity. It provides detailed chemical profiling, creating unique "fingerprints" valuable for quality control and standardization. This technique enables accurate quantification, aiding in understanding potency and correlating bioactive compounds with therapeutic effects. Additionally, GC-MS supports quality assurance in herbal medicine, ensuring consistency and safety. Its comprehensive analysis of plant compositions advances both natural product research and the discovery of new bioactive compounds. The molecular docking workflow was meticulously executed through sequential stages of protein refinement, ligand preparation, and computational docking to delineate the binding interactions between the bioactive constituents of *Grateloupia lithophila* and gastric cancer–associated protein targets. The present investigation aims to comprehensively characterize the phytochemical constituents of the ethanolic extract of *Grateloupia lithophila* (EEGL) through GC–MS analysis and to elucidate their molecular interactions with key protein targets implicated in gastric carcinogenesis. The study further employs *in silico* docking to assess binding affinities, pharmacokinetic profiles, and potential mechanistic pathways of selected metabolites. Particular emphasis is placed on cholesta-based derivatives, which have demonstrated both anticancer and anti-*H. pylori* activities, thereby offering a promising dual therapeutic approach against gastric malignancies [16]. This integrative strategy not only expands the chemotaxonomic understanding of *G. lithophila* but also paves the way for its development as a marine-derived source of multifunctional anti gastric cancer agents.

Material and Methods

Collection of plant material

The Red algae of *Grateloupia lithophila Borgesen* were collected from, Pudhumadam in Ramanathapuram district in Tamil Nadu, India during the month of March 2025. The algal samples were carefully rinsed with seawater followed by freshwater to remove epiphytes and sand particles. Samples were shade dried and stored in airtight containers. The plant was authenticated by marine scientist *Dr.veeraguranathan,* chief scientist of CSIR-CMCRI, Mandapam Camp, Ramanathapuram district and confirmed as *Grateloupia lithophila Borgesen* (Rhodophyta:

Halymeniaceae). The coarsely powdered *Grateloupia lithophila* was subjected to extraction with ethanol in the proportion of 1: 4 and the resultant residue, designated as the ethanolic extract of *Grateloupia lithophila* (EEGL).

GC-MS chromatography

EEGL was analysed via GC-MS with high-speed settings for suction and injection, using a 0.3-second dwell time and a 6 µL washing volume. The GC-2010 system was configured with an oven temperature program from 50°C to 280°C, a 250°C injection temperature, and a split ratio of 10.0. Linear velocity flow control was used, with a 68.1 kPa pressure and a 1.20 mL/min column flow. The ion source and interface temperatures were 200°C and 250°C, respectively, with a solvent cut time of 3.5 minutes. Detector gain was set relative to the tuning result. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Molecular docking

Required softwares

- Discovery studio visualize - To visualize and refine the enzyme.
- Chem. sketch or chem. Draw - for drawing a structure and generate the smiles.
- Online smile translator - to convert ligand to .pdb file.

Supporting softwares

- Python Cygwin, Mgl tools (auto dock & auto grid), Python – cygwin - mgl tool

Sequel:

Identification of phytoconstituent in EEGL extarct by GAS chromatography – Mass spectroscopy

The GC-MS analysis of the ethanolic extract of *Grateloupia lithophila* (EEGL) revealed a chemically diverse profile comprising terpenoids, fatty acids, steroids, esters, alkaloids, polyphenols, and long-chain hydrocarbons. A total of approximately 68 bioactive compounds were identified, characterized by molecular formula, molecular weight, retention time, and peak area. Spectral matching with the NIST library confirmed the chemical diversity and pharmacological potential of the extract. The intricacies of study were observed, which are illustrated in fig.no.1 and represented in table no.1 to 5.

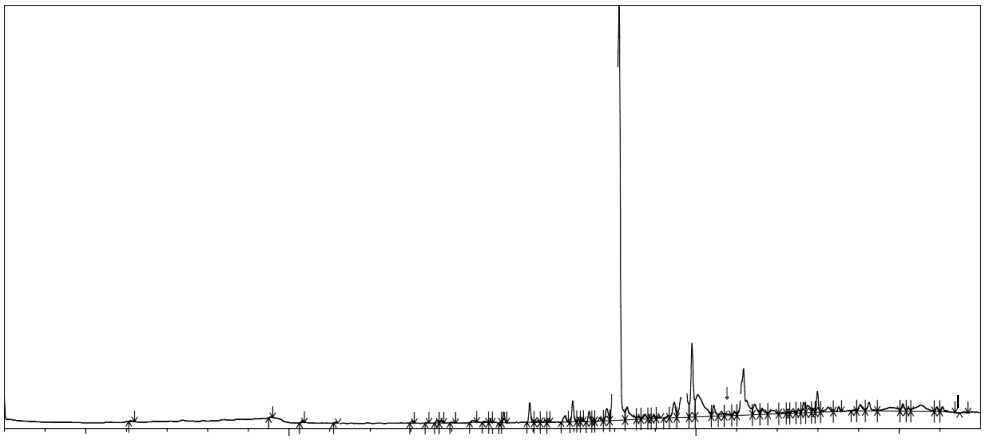


Figure 1: GC-MS chromatogram of EEGL

Table 1: Terpenoid class of compounds in EEGL

Compound	Subclass	Mol. Formula	M.W	R.T	Area	Fig.
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	Sesquiterpene lactone	$C_{11}H_{16}O_2$	180.24	13.386	109878	2(a)
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	Terpenoid derivative	$C_{11}H_{16}O_3$	196.24	16.115	472,710	2(b)
2-Pentadecanone, 6,10,14-trimethyl-	Terpenoid	$C_{18}H_{36}O$	268.48	16.791	1,793,635	2(c)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol derivative)	Diterpenoid	$C_{20}H_{40}O$	296.53	17.190	239,131	
Phytol	Diterpenoid	$C_{20}H_{40}O$	296.53	19.470	4,832,101	2(d)
Ageratriol	sesquiterpene	$C_{15}H_{24}O_3$	252.35	20.800	907,685	2(e)
Supraene	Triterpenoid	$C_{30}H_{50}$	410.72	25.227	920,745	2(f)
Cholesta-4,6-dien-3-ol, (3β-)	Sterol (Triterpenoid)	$C_{27}H_{44}O$	384.64	25.924	895,257	2(g)
Neophytadiene	Diterpenoid	$C_{20}H_{38}$	278.52	19.398	119012	2(i)
Heptadecane, 2,6,10,15-tetramethyl	Diterpenoid	$C_{21}H_{44}$	296.57	14.244	86945	
Methyl 7α-hydroxy-3α-methoxy-5β-cholanoate	Steroid (triterpenoid)	$C_{26}H_{44}O_4$	420	30.003	299636	2(h)
Methyl 3α-hydroxy-7α-methoxy-5β-cholanoate	Steroid (triterpenoid)	$C_{26}H_{44}O_4$	420	30.007	299639	

The structures of the most biologically important compounds, corresponding to those with the highest area, are represented in Fig.no 2(a)-2(h).

Table 2: Alkaloid class of compounds in EEGL

These compounds structures were represented in figure 3(a) - 3(d)

Compound name	Subclass	Molecular Formula	M.W	R. (min)	Area (%)	Fig.
Lidocaine	Synthetic Alkaloid (Aminoamide class)	$C_{14}H_{22}N_2O$	234	17.375	2090058	3 (a)
Benzyl ephedrine	Phenethylamine Alkaloid	$C_9H_{13}NO$	151	22.260	0.07	3 (b)
Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate	Quaternary Ammonium Alkaloid	$C_{24}H_{38}N_2O_2$	394	22.880	0.37	3 (c)
[1,3]Diazepan-2,4-dione	Alkaloid-like Nitrogen Heterocycle	$C_7H_{10}N_2O_2$	142	10.320	0.22	3 (d)

Table 3: Polyphenol class of compounds in EEGL

Compounds structures were illustrated in **fig no: 4(a) - 4(c)**

Compound name	Subclass	Molecular Formula	M.W	R.T (min)	Area (%)	Fig.
2,4-Di-tert-butylphenol	Alkylated phenol	$C_{14}H_{22}O$	206	13.020	0.24	4 (a)
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	Benzofuranone lactone	$C_{11}H_{16}O_2$	180	13.386	0.07	4 (b)
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	Benzofuranone lactone	$C_{11}H_{16}O_3$	196	16.115	0.16	
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Spiroketone derivative	$C_{18}H_{22}O_3$	286	17.480	0.07	
9-(4-Methoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-2H-xanthene-1,8-dione	Xanthene derivative	$C_{23}H_{28}O_4$	370	26.606	0.12	4(c)

Table 4: Fatty acid class of compounds in EEGL

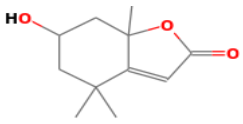
Compounds used in cancer treatment that structures were presented in **fig.no: 5(a) - 5(s)**

Compound name	Subclass	Molecular Formula	M.W	R.T (min)	Area (%)	Fig.
n-Decanoic acid (Capric acid)	Saturated fatty acid	$C_{10}H_{20}O_2$	172	11.144	0.13	5 (a)
Dodecanoic acid (Lauric acid)	Saturated fatty acid	$C_{12}H_{24}O_2$	200	13.637	0.30	5 (b)
Fumaric acid, ethyl 2-methylallyl ester	Ester of fumaric acid	$C_7H_{10}O_4$	174	13.737	0.25	
Azelaic acid	Dicarboxylic acid	$C_9H_{16}O_4$	188	14.513	0.21	5 (c)
Oleic Acid	Monounsaturated fatty acid	$C_{18}H_{34}O_2$	282	14.801	0.12	5 (d)
8-Pentadecanone	Ketone	$C_{15}H_{30}O$	226	14.954	0.09	
Eicosyl acetate	Fatty acid ester	$C_{22}H_{44}O_2$	340	15.185	0.05	
Tetradecanoic acid (Myristic acid)	Saturated fatty acid	$C_{14}H_{28}O_2$	228	15.917	1.97	5 (e)
Pentadecanoic acid	Saturated fatty acid	$C_{15}H_{30}O_2$	242	16.973	2.11	5 (f)
Hexadecanoic acid, methyl ester (Methyl palmitate)	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270	17.636	0.36	
6-Pentadecenoic acid, 13-methyl-, (6Z)-	Unsaturated fatty acid	$C_{16}H_{30}O_2$	254	17.808	1.24	

n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid	$C_{16}H_{32}O_2$	256	18.112	41.74	5 (g)
Hexadecanoic acid, ethyl ester (Ethyl palmitate)	Fatty acid ethyl ester	$C_{18}H_{36}O_2$	284	18.311	1.29	
9,12-Octadecadien-1-ol, (Z,Z)- (Linoleyl alcohol)	Unsaturated fatty alcohol	$C_{18}H_{34}O$	266	18.585	0.25	
Oleic Acid	Monounsaturated fatty acid	$C_{18}H_{34}O_2$	282	18.749	0.43	
Tetracosanoic acid, methyl ester (Methyl lignocerate)	Fatty acid methyl ester	$C_{25}H_{50}O_2$	370	18.855	0.08	5 (h)
Heptadecanoic acid (Margaric acid)	Saturated fatty acid	$C_{17}H_{34}O_2$	270	18.958	0.31	5 (i)
9-Octadecenoic acid, methyl ester, (E)- (Methyl oleate)	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296	19.316	0.25	
Oleic Acid	Monounsaturated fatty acid	$C_{18}H_{34}O_2$	282	19.708	13.99	
Octadecanoic acid (Stearic acid)	Saturated fatty acid	$C_{18}H_{36}O_2$	284	19.908	7.38	5 (j)
cisZ-11,12-Epoxytetradecan-1-ol	Epoxy fatty alcohol	$C_{14}H_{28}O_2$	228	20.050	2.22	5 (k)
(9E,11E)-Octadecadienoic acid (Conjugated linoleic acid)	Conjugated fatty acid	$C_{18}H_{32}O_2$	280	20.441	1.09	5 (l)
Palmitoyl chloride	Fatty acyl chloride	$C_{16}H_{31}ClO$	270	20.656	0.24	
Dimethylaminoethyl palmitate	Fatty acid amine ester	$C_{20}H_{43}NO_2$	317	20.962	0.35	
7Z,10Z,13Z,16Z,19Z-Docosapentaenoic acid (DPA)	Polyunsaturated fatty acid (ω-3)	$C_{22}H_{34}O_2$	346	21.172	4.63	5(m)
Eicosanoic acid (Arachidic acid)	Saturated fatty acid	$C_{20}H_{40}O_2$	312	21.632	0.61	5 (n)
Oleoyl chloride	Fatty acyl chloride	$C_{18}H_{33}ClO$	296	22.143	0.13	
Glycerol 1-palmitate (Monopalmitin)	Monoacyl glycerol	$C_{19}H_{38}O_4$	330	22.379	0.21	
1,3-Dipalmitin, TMS derivative	Diacyl glycerol derivative	$C_{35}H_{70}O_5$	562	22.531	0.33	
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Glyceryl monopalmitate)	Monoacyl glycerol ester	$C_{19}H_{38}O_5$	346	22.655	1.04	5 (o)

Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (Glyceryl dipalmitate)	Diacyl glycerol	$C_{35}H_{68}O_5$	560	23.254	0.46	
Oleoyl chloride	Fatty acyl chloride	$C_{18}H_{33}ClO$	296	24.045	0.62	
1-Decanoyl-3-dodecanoylglycerol	Diacyl glycerol	$C_{25}H_{50}O_4$	418	25.533	0.63	5 (p)
Heptadecane	Alkane hydrocarbon	$C_{17}H_{36}$	240	15.279	1.07	
1-Nonadecene	Long-chain alkene	$C_{19}H_{38}$	266	16.290	0.22	
Hexadecane	Alkane hydrocarbon	$C_{16}H_{34}$	226	16.362	0.10	
2-Pentadecanone, 6,10,14-trimethyl-	Ketone (isoprenoid-derived)	$C_{18}H_{36}O$	268	16.791	0.64	
1-Tetracosene	Long-chain alkene	$C_{24}H_{48}$	336	21.450	1.05	5 (q)
Octacosane	Long-chain alkane	$C_{28}H_{58}$	394	22.752	0.73	
Tetratetracontane	Very long-chain alkane	$C_{44}H_{90}$	618	23.512	0.48	5 (r)
Nonacosane	Long-chain alkane	$C_{29}H_{60}$	404	24.260	0.90	5 (s)
Tetratetracontane	Very long-chain alkane	$C_{44}H_{90}$	618	25.091	0.75	
Hexatriacontane	Long-chain alkane	$C_{36}H_{74}$	506	26.066	0.48	

Terpenoid class of compounds



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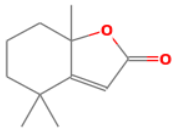
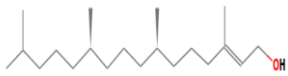


2(b)

2(c)



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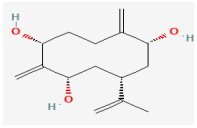

2(e)

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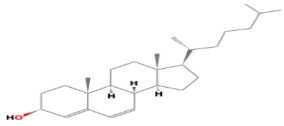

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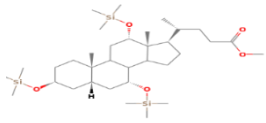
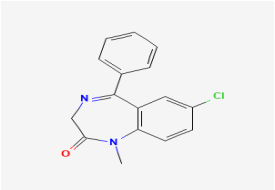


2(h)

Alkaloid class of compounds



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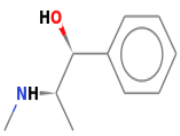

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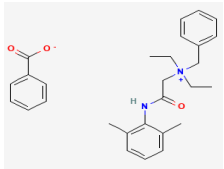

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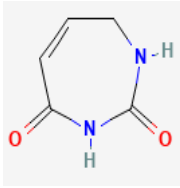
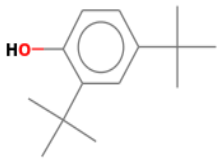


3(d)

Polyphenol class of compounds



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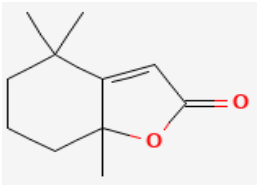

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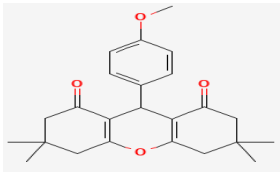


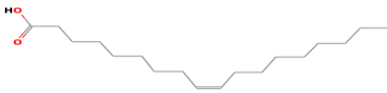
4(c)

Fatty acid class of compounds

5(a)

5(b)

5(c)



5(d)

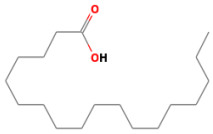
5(e)

5(f)

5(g)

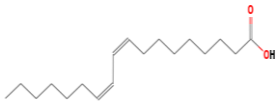
5 (h)

5(i)

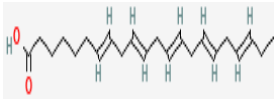


5 (j)

5(k)



5(l)



5 (m)

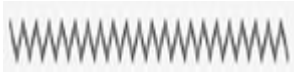
5(n)

5(o)

5(p)

5(q)

5(r)



5(s)

Table 5: Biological activity of selected compounds

Compounds	Common Name	Biological Activity	References
Benzeneacetic acid	**Phenylacetic acid**	Anti-inflammatory, antitumor, antimicrobial.	[19,28]
n-Decanoic acid	**capric acid**	Antimicrobial, anti-inflammatory, anticancer potential.	[19,28]
2,4-Di-tert-butylphenol		Antioxidant, antimicrobial, cytotoxic.	[15,27]
Phytol	**phytol**	Anti-inflammatory, antioxidant, antimicrobial	[19,28]
Ageratriol	**Ageratriol**	Anti-proliferative, anti-ulcer, anti-inflammatory and anti-breast cancer.	[19]

Supraene	**squalene**	Immune stimulant, anaesthetic and wound healing.	[23]
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-(Dihydroactinidiolide)	**Dihydro actinidiolide**	Antioxidant, anti-inflammatory, anticancer, anti-bacterial.	[15,27]
Fumaric acid, ethyl 2-methylallyl ester	**Fumaric acid**	Anti-inflammatory, anticancer, & antioxidant.	[19]
Azelaic acid	**Azelaic acid**	Antimicrobial, anti-inflammatory, antitumor potential.	[19,28]
Oleic Acid	**Oleic Acid**	Anti-inflammatory, cardioprotective, antimicrobial, & anticancer potential.	[19,28]
8-Pentadecanone	**Caprylone**	Antimicrobial, wound healing & antioxidant.	[15]
Heptadecane	**n-Heptadecane**	Antimicrobial, antioxidant & anti-inflammatory.	[15,27]
Tetradecanoic acid	**Myristic acid**	Antimicrobial, antiviral, anti-inflammatory, & anticancer potential.	[19,28]
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	**Loliolide**	Antioxidant, anti-inflammatory	[15,19]
Pentadecanoic acid	**Pentadecyclic acid**	Anti-inflammatory, anticancer potential.	[19,28]
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	**phytol**	Antioxidant, antidiuretic, anti-inflammatory, & anticancer.	[19,28]
Hexadecanoic acid, methyl ester	**Methyl palmitate**	Anti-inflammatory, hepatoprotective, antioxidant, anticancer potential.	[19,28]
n-Hexadecanoic acid	**Palmitic acid**	Antimicrobial, antioxidant, anticancer potential.	[19,28]
9,12-Octadecadien-1-ol, (Z,Z)- (Linoleyl alcohol)	**Linoleyl alcohol**	Antioxidant, hypolipidemic, anticancer potential.	[19,28]
9-Octadecenoic acid, methyl ester, (E)-(Methyl oleate)	**Methyl elaidate**	Anti-inflammatory, antifungal, anti-microbial & antioxidant.	[19,28]
Octadecanoic acid	**Stearic acid**	Antimicrobial, antiviral, anti-inflammatory, anticancer potential.	[19,28]
cisZ-11,12-Epoxytetradecan-1-ol		Antimicrobial, cytotoxic.	[19]

(9E,11E)-Octadecadienoic acid (Conjugated linoleic acid)	**Rumenic acid , mangold's acid & iso linoleic acid**	Anti-carcinogenic, anti atherogenic, anti-obesity, antiinflammatory,	[19,20]
7Z,10Z,13Z,16Z,19Z-Docosapentaenoic acid (DPA)	**Clupanodonic acid**	Anti-inflammatory, anticancer, cardioprotective.	[19,20]
Cyclodecane, octyl-	**cyclodecane**	Antimicrobial, anti-inflammatory.	[15]
Octacosane	**n- Octacosane**	Antimicrobial, antioxidant.	[15,27]
1-Decanoyl-3-dodecanoylglycerol	**diacylglycerol**	Antimicrobial, anti-inflammatory.	[19]
Cholesta-4,6-dien-3-ol, (3.beta.)-		Anticancer, anti-inflammatory.	[16,17,26,29]
Hexatriacontane	**Hexatriacontane**	Antioxidant, cytotoxic, antimicrobial.	[15,27]
9-(4-Methoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-2H-xanthene-1,8-dione	**Xanthene dione**	Antioxidant, antimicrobial & cytotoxic.	[19]
1,2-Benzene di carboxylic acid, dioctyl ester	**Di n-octyl phthalate**	Antioxidant, anti- tubercular, cytotoxic and anti-inflammatory	[19,28]
Heptadecane, 2,6,10,15-tetramethyl	**Tetramethyl heptadecane**	Antioxidant, cytotoxic and anti-inflammatory	[15,27]
Methyl 7α-hydroxy-3α-methoxy-5β-cholanoate	**Cholanoic acid**	Antioxidant, cytotoxic and anti-inflammatory	[23,26]
Methyl 3α-hydroxy-7α-methoxy-5β-cholanoate	**Cholanoic acid**	Antioxidant, cytotoxic and anti-inflammatory	[23,26]

The most abundant constituent was benzene dicarboxylic acid dioctyl ester, representing 86.78% of the chromatogram. Major fatty acids included n-hexadecanoic acid (palmitic acid) with 47.87% at peak 27 and a retention time of 18.112 minutes, oleic acid with 12.61% at peak 36 and 19.708 minutes, octadecanoic acid with 5.17% at peak 37, and 7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid with 5.96% at peak 43 and 21.172 minutes. Additional fatty acid derivatives included hexadecanoic acid ethyl ester at 1.56% (peak 28) and pentadecanoic acid at 1.11% (peak 20). Terpenoids were prominently represented by phytol at 1.29% (peak 35, 19.470 minutes), 2-pentadecanone, 6,10,14-trimethyl- at 1.79%, and ageratriol at 0.91%, which is reported here for the first time in G. lithophila. Steroidal compounds included methyl 7α-hydroxy-3α-methoxy-5β-cholanoate at 1.46% (30.003 minutes) and cholesta-4,6-dien-3-ol (3β-) at 0.90% (25.924 minutes), along with supraene at 0.92%, also identified for the first time in this species. Among alkaloids, lidocaine was the dominant peak at 2.09% (17.375 minutes), while benzylephedrine was only detected at 0.07%. Polyphenolic compounds, including benzofuranone

and xanthene derivatives, were observed in moderate amounts, suggesting antioxidant potential. Other notable constituents included cis-Z,11,12-epoxytetradecane-1-ol at 4.84% (peak 38), hexadecenal at 1.29% (peak 60), 1-decanoyl-3-dodecanoyl glyceryl at 1.86% (peak 63), and 1-tetracosene at 1.14% (peak 44). Compounds at peaks 1, 2, 6, 9, 11, 12, 13, 18, 20, 24, 31, 33, and 48 were detected only in trace amounts. Notably, several metabolites are reported here for the first time in this genus, including ageratriol**,** supraene**,** benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate**,** dimethylaminoethyl palmitate**,** 1-decanoyl-3-dodecanoylglycerol**,** cyclodecane, octyl-**, and a** xanthene derivative**.** These novel findings broaden the chemotaxonomic spectrum of *G. lithophila* and highlight its potential as a source of unique bioactive compounds.

Table 6: Lipinski's properties of the selected bioactive compounds from extracts of EEGL

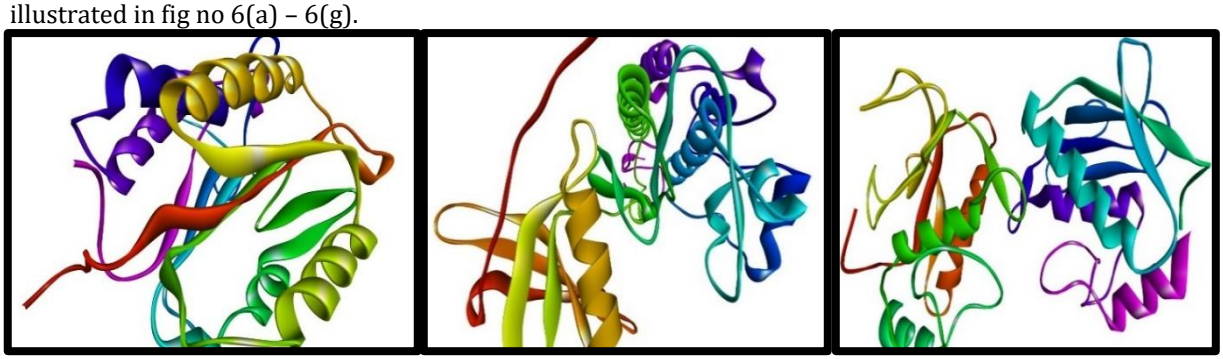
Name of the compound	Mol.wt (g/mol)	Log P (<500 Kd)	H-bond donor (<5)	H- bond acceptor (<10)
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	180.24	2.37	0	2
Tetradecanoic acid	228.37	3.69	1	2
Pentadecanoic acid	242.40	3.94	1	2
Lidocaine	234.34	2.38	1	2
6-Pentadecenoic acid, 13-methyl-, (6Z)-	282.46	4.57	1	2
Heptadecanoic acid	270.45	4.44	1	2
9-Octadecenoic acid, methyl ester, (E)-	296.49	4.80	0	2
Phytol	296.53	5.25	1	1
Dimethylaminoethyl palmitate	327.55	4.25	0	3
Benzylephedrine	255.35	3.16	1	2
Cholesta-4,6-dien-3-ol, (3.beta.)-	384.64	6.23	1	1
5,6-Dihydro-5-methyluracil	272.49	1.45	0	2
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	180.24	2.37	0	2
Decyl propylphosphonofluoridate	184.15	0.98	0	4
2-(Diethylamino)ethyl 4-amino-2-	341.66	3.24	1	3
1,2-Benzenedicarboxylic acid,dioctyl ester	390.56	5.24	0	4
methyl 7alpha-hydroxy-3alpha-methoxy-5beta-cholanoate	420.63	4.29	1	4
methyl 3alpha-hydroxy-7alpha-methoxy-5beta-cholanoate	420.63	4.29	1	4
Ageratriol	252.35	1.66	3	3

Table 7: Molecular docking score for bioactive compounds of EEGL

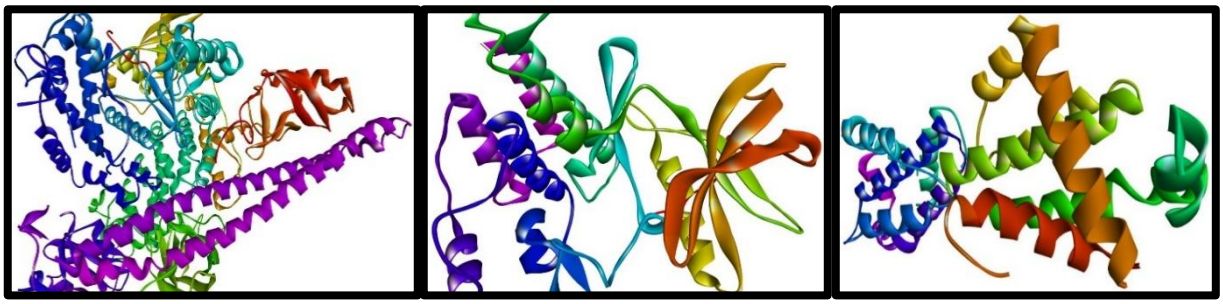
Si. No	List of Compounds	Binding Score with HDR-GENE						
		1PAU	**1M17**	**1GKC**	**3HHM**	**3WZE**	**4LVT**	**5IKR**
1	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	-5.7	-5.9	-5.5	**-6.4**	**-6.3**	-5.8	**-6.4**
2	Tetradecanoic acid	-4.7	-4.8	-5.8	-4.5	**-6.2**	-4.8	-5.2
3	Pentadecanoic acid	-4.5	-5.1	**-6.3**	-5.5	-5.8	-4.5	-4.5
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-5.2	-4.5	**-6.7**	-5.8	**-7.3**	-5.7	-5.4
5	Lidocaine	-5.1	-4.7	-5.4	**-6.6**	-6	-5.4	-5.8
6	6-Pentadecenoic acid, 13-methyl-, (6Z)-	-5.3	-5.6	**-6.7**	-5.7	**-7**	-5.3	-5.4
7	Heptadecanoic acid	-4.7	-4.9	**-6.2**	-4.9	**-6.4**	-4.8	**-6.1**
8	9-Octadecenoic acid, methyl ester, (E)-	-4.8	-4.6	**-6.3**	-5.6	**-6.8**	-5.1	-5.5
9	Phytol	-5.0	-5.9	**-6.6**	-5.6	-5.8	-5.5	-5.4
10	Dimethylaminoethyl palmitate	-4.8	-3.8	-5.5	-5	**-6.6**	-5.4	-5.2
11	Benzylephedrine	-5.0	-4.1	**-7.8**	-6	**-6.4**	**-7.1**	**-7.4**
12	Cholesta-4,6-dien-3-ol, (3.beta.)-	**-7.1**	**-7.9**	**-8.3**	**-8.1**	**-9.7**	**-8.2**	**-9.3**
13	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	-5.6	-6.0	-5.1	**-6.4**	-6	**-6.4**	**-6.4**
14	Decyl propylphosphonofluoridate	-4.3	-4.8	-5	-5.1	-5.4	-4.6	-5.3
15	1,2-Benzenedicarboxylic acid,dioctyl ester	-5.9	-4.8	**-7.8**	-6	**-6.4**	-5	-6
16	methyl 7alpha-hydroxy-3alpha-methoxy-5beta-cholanoate	**-7.1**	**-8.1**	**-7.6**	**-8.1**	**-8.8**	**-7.7**	**-8.4**
17	methyl 3alpha-hydroxy-7alpha-methoxy-5beta-cholanoate	**-6.7**	**-8.4**	**-7.8**	**-8.4**	**-8.6**	**-7.4**	**-9.8**
18	**Ageratriol**	-5.8	**-7.3**	**-6.4**	**-7.4**	**-7.1**	**-6.4**	**-7.7**
Standard Drug								
19	5-Fluorouracil	-4.2	-4.8	**-6.1**	-5.8	-5.1	-4.8	-5.9

Structure of target proteins involved in molecular docking

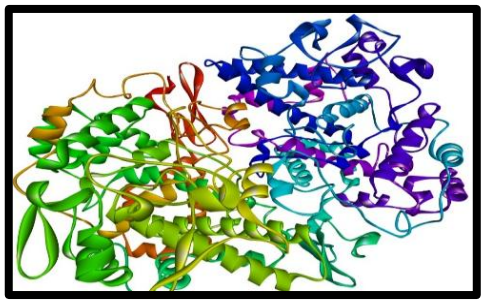
Structures of Proteins implicated in stomach cancer progression used for molecular docking analysis were illustrated in fig no 6(a) – 6(g).



6(a) **6(b)** **6(c)**



6(d) **6(e)** **6(f)**



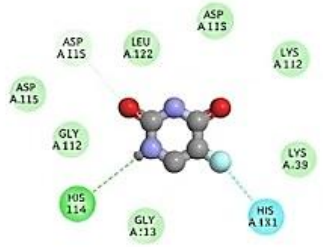
6(g)

a) **1PAU**
b) **1M17**
c) **1GKC**
d) **3HHM**
e) **3WZE**
f) **4LVT**
g) **5IKR**

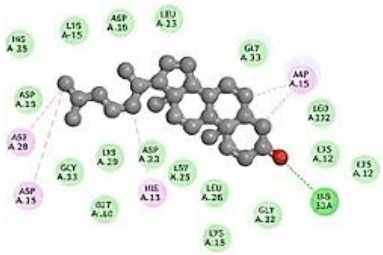
Binding affinity of target and bioactive compounds

Molecular docking was performed to elucidate the interactions of eighteen bioactive compounds from *Grateloupia lithophila* with seven protein targets implicated in stomach cancer progression, including 1PAU, 1M17, 1GKC, 3HHM, 3WZE, 4LVT, and 5IKR. Lipinski's rule of five analysis confirmed that most of the selected phytoconstituents exhibited acceptable molecular weights, Log P values, and hydrogen-bonding parameters, suggesting good oral bioavailability and drug-likeness. The three-dimensional structures of the target proteins employed in the docking experiments are illustrated in Fig. 79(a–g), while the comprehensive Lipinski parameters and docking scores are provided in Table no.6 and Table no.7, respectively.

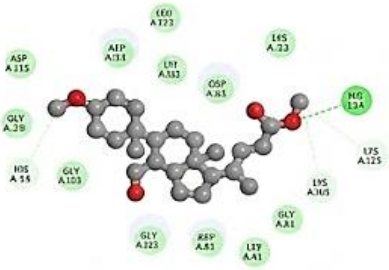
PLATE A: BIKR - BIOACTIVE COMPOUNDS



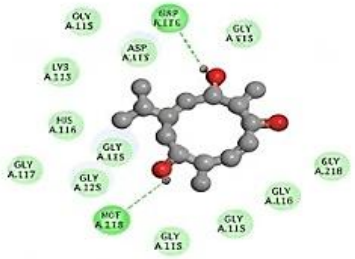
STANDARD 5-FU



Cholesta-4,6-dien-3-ol, (3-beta)-

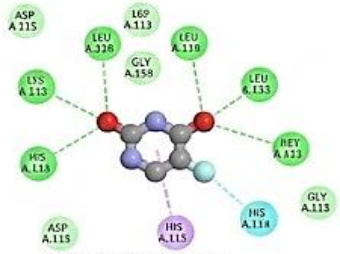


methyl 7alpha-hydroxy-3alpha-methoxy-5beta-cholanoate

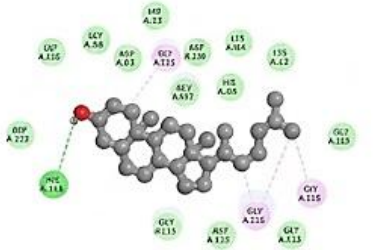


Agaruorol

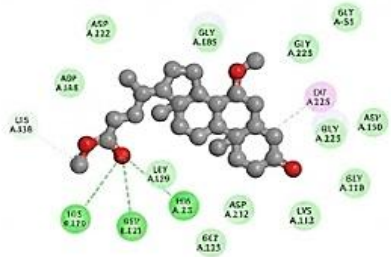
Plate B: SHHM – Bioactive Compounds



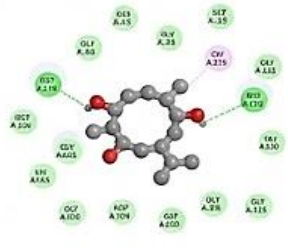
STANDARD 5-FU



Cholesta-4,6-dien-3-ol, (3-beta)-



methyl 7alpha-hydroxy-3alpha-methoxy-5beta-cholanoate



Agarurrol

The docking results revealed notable variation in binding affinities across the different phytoconstituents.Among the evaluated molecules, cholesta-4,6-dien-3-ol (3β) exhibited the most potent binding affinities across all targets, with binding energies ranging from −7.1 to −9.7 kcal mol^{-1}. Close affinities were observed for methyl 7α-hydroxy-3α-methoxy-5β-cholanoate (−7.1 to −8.8 kcal mol^{-1}) and methyl 3α-hydroxy-7α-methoxy-5β-cholanoate (−6.7 to −9.8 kcal mol^{-1}), reflecting strong interactions with all seven receptor proteins. Ageratriol also showed appreciable binding energies (−5.8 to −7.7 kcal mol^{-1}), followed by benzylephedrine (−4.1 to −7.8 kcal mol^{-1}) and

1,2-benzenedicarboxylic acid dioctyl ester (−4.8 to −7.8 kcal mol^{-1}).Moderate binding affinities were recorded for 2(4H)-benzofuranone derivatives (−5.1 to −6.4 kcal mol^{-1}), phytol (−5.0 to −6.6 kcal mol^{-1}), 6-pentadecenoic acid (−5.3 to −7.0 kcal mol^{-1}), and lidocaine (−4.7 to −6.6 kcal mol^{-1}). Fatty acids such as tetradecanoic (−4.5 to −6.2 kcal mol^{-1}), pentadecanoic (−4.5 to −6.3 kcal mol^{-1}), and heptadecanoic acid (−4.7 to −6.4 kcal mol^{-1}) displayed comparable interactions. Dimethylaminoethyl palmitate (−3.8 to −6.6 kcal mol^{-1}) and decyl propylphosphonofluoridate (−4.3 to −5.4 kcal mol^{-1}) showed relatively weaker affinities.

The reference chemotherapeutic agent 5-fluorouracil exhibited comparatively lower binding scores ranging from −4.2 to −6.1 kcal mol^{-1}, underscoring the superior binding potential of several *G. lithophila*-derived metabolites. Overall, the distribution of binding energies highlighted cholesta-4,6-dien-3-ol (3β) as the most promising inhibitor across all targets, followed by methyl 7α-hydroxy-3α-methoxy-5β-cholanoate, methyl 3α-hydroxy-7α-methoxy-5β-cholanoate, and ageratriol, which collectively demonstrated strong molecular interactions against stomach cancer-associated proteins.

Deliberation

The GC–MS profile of the ethanolic extract of *Grateloupia lithophila* revealed a metabolically rich assemblage of compounds comprising fatty acids, esters, terpenoids, steroids, alkaloids, and polyphenols. The coexistence of both saturated and unsaturated fatty acids indicates the nutraceutical potential of the extract, particularly for anti-inflammatory and cardioprotective applications [27]. Comparative analysis with previous GC–MS studies on other *Grateloupia* species demonstrated consistent patterns of bioactive metabolites. Fatty acids such as palmitic and oleic acid, along with sterols like methyl 7α-hydroxy-3α-methoxy-5β-cholanoate, have been consistently detected in *G. lithophila* and its congeners [9,15,26]. These compounds are widely recognized for their antioxidant, antimicrobial, anti-inflammatory, and anticancer properties, thereby substantiating the pharmacological potential of the extract [28,29].

Phytochemical parallels among *Grateloupia* species also reinforce the chemotaxonomic relevance of these metabolites. Ethanolic extracts of *G. livida* revealed compounds such as tetratricontane, pentatricontane, stigmasterol, oleic acid, dodecanoic acid, and phytol [15], whereas methanolic extracts of *G. doryphyra* and aqueous extracts of *G. filicina* exhibited comparable fatty acid and sterol profiles [9,27]. The persistence of terpenoids, sterols, and fatty acids across species underscores conserved biosynthetic mechanisms and ecological adaptations, supporting their role as taxonomically stable biochemical markers [9,26,28].

Additionally, terpenoids such as phytol and ageratriol, identified in measurable concentrations in EEGL, are associated with cytotoxic, antimicrobial, and immunomodulatory activities, validating the pharmacological significance of this species [21,22]. A key outcome of this investigation was the identification of novel compounds in *G. lithophila* including ageratriol, supraene, 1-decanoyl-3-dodecanoyl glyceryl, dimethylaminoethyl palmitate, benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate, octylcyclodecane, and a xanthene derivative none of which have previously been reported in *Grateloupia* species. The discovery of these unique metabolites expands the phytochemical spectrum of the genus, suggesting unexplored metabolic pathways and novel pharmacological targets [25,30]. Collectively, the chemical diversity and abundance of these metabolites affirm the potential of *G. lithophila* as a promising source of nutraceutical and therapeutic leads.

Molecular docking was employed as a crucial computational approach to elucidate the binding affinities, interaction profiles, and inhibitory mechanisms of the identified phytoconstituents against gastric cancer–associated protein targets [1,2,4]. In this study, bioactive compounds identified from the ethanolic extract were

docked against seven key protein targets are 1PAU, 1M17, 1GKC, 3HHM, 3WZE, 4LVT, and 5IKR linked to gastric carcinogenesis. Of the 76 compounds initially identified through GC–MS profiling, 19 were prioritized through network pharmacology for their predicted relevance to gastric cancer signaling pathways, and 17 satisfied Lipinski's rule of five criteria. Among these, three bioactive molecules of cholesta-4,6-dien-3-ol (3β), methyl 7α-hydroxy-3α-methoxy-5β-cholanoate, and ageratriol demonstrated the highest docking affinities. The chemotherapeutic agent 5-fluorouracil (5-FU) was used as a reference standard for comparative analysis [18,23]. The most significant interactions were observed with 5IKR (human epidermal growth factor receptor kinase domain) and 3HHM (Bcl-2 family protein), where cholesta-4,6-dien-3-ol and methyl cholanoate derivatives exhibited highly negative docking energies (−9.3 and −8.4 kcal.mol^{-1}, respectively), indicating strong inhibitory potential [12,13]. The interaction analysis revealed multiple hydrogen bonds, hydrophobic interactions, and π–π stacking within the active sites of these proteins, suggesting stable and energetically favorable binding conformations. These findings imply potential suppression of receptor phosphorylation in 5IKR and inhibition of anti-apoptotic activity in 3HHM, two critical mechanisms implicated in gastric tumor proliferation and chemoresistance [14,17].

Interestingly, cholesta derivatives are also documented to exhibit potent antibacterial activity against *Helicobacter pylori*, a pathogen strongly associated with gastric carcinoma, thereby reinforcing their therapeutic significance in preventing stomach cancer [10,30]. These results are consistent with earlier reports on *G. filicina* and *G. turuturu*, where fatty acid and terpenoid constituents demonstrated inhibitory effects on tyrosine kinase and Bcl-2 proteins, leading to apoptosis and cell cycle arrest [26,31,32]. The steroidal derivative cholesta-4,6-dien-3-ol has also been recognized for its strong hydrophobic complementarity toward kinase receptor pockets, corroborating its predicted binding behavior in this study [17,29]. Similarly, methyl 7α-hydroxy-3α-methoxy-5β-cholanoate showed comparable affinity to marine steroidal compounds known to disrupt PI3K/Akt signaling cascades [16,24]. The sesquiterpenoid ageratriol exhibited moderate yet meaningful binding to apoptosis-regulating proteins, consistent with prior studies reporting its activity as an apoptotic modulator [22].

Comparative docking with 5-fluorouracil further reinforced the pharmacological relevance of these compounds. Although 5-FU is an established chemotherapeutic drug targeting thymidylate synthase and RNA pathways, its docking scores in this study were comparatively lower, indicating that *G. lithophila* derived metabolites may act through alternative or synergistic mechanisms particularly kinase inhibition and apoptosis induction which may enhance therapeutic efficacy when used in conjunction with conventional chemotherapy [18,20,33].

Integration of these docking results with experimental findings from the green-synthesized silver nanoparticles (AgNPs-AEGL) provides a more comprehensive mechanistic understanding. The same phytoconstituents responsible for docking activity likely serve as reducing and stabilizing agents during nanoparticle synthesis, improving stability, cellular uptake, and bioavailability [8,34]. Such dual functionality could explain the enhanced cytotoxic and pro-apoptotic activity observed against AGS gastric carcinoma cells [11,34].

Collectively, the docking and network pharmacology outcomes highlight a polypharmacological mode of action, wherein *G. lithophila* metabolites interact with multiple oncogenic signaling pathways rather than a single molecular target. This multi-target interference is particularly advantageous in mitigating drug resistance and complex tumor signaling in gastric cancer [19,33,35]. The high docking affinities of sterol and cholanoate derivatives also suggest that ethanolic extraction effectively recovers medium-polar ligands with favorable pharmacokinetic profiles [28,29]. Future studies involving molecular dynamics simulations, free energy analyses,

and receptor inhibition assays are essential to validate these computational predictions and translate *G. lithophila* derived compounds into potential nanotherapeutic candidates against gastric malignancies [14,36].

Conclusion

The present study elucidates the phytochemical and molecular landscape of *Grateloupia lithophila*, underscoring its prominence as a metabolically versatile red alga with remarkable pharmacological potential. The GC–MS profile revealed a diverse array of fatty acids, sterols, and terpenoids, several of which exhibited strong binding affinities toward pivotal protein targets implicated in gastric carcinogenesis. Notably, cholesta-based derivatives displayed dual bioactivities, combining potent anticancer efficacy with significant antibacterial action against *Helicobacter pylori* a primary etiological agent in gastric cancer progression [10,17,26,30]. These findings collectively affirm the therapeutic relevance of *G. lithophila* metabolites as promising scaffolds for the development of multifunctional anticancer and antimicrobial agents.

Future perspectives

Future investigations should emphasize *in vitro* and *in vivo* validation of these lead compounds to substantiate their mechanistic pathways and therapeutic selectivity [14,36]. The integration of molecular dynamics simulations, binding free energy analyses, and ADMET profiling could provide deeper insight into their pharmacokinetic and pharmacodynamic attributes [6,24]. Moreover, the incorporation of *G. lithophila*–derived bioactives into nanocarrier-based drug delivery systems may enhance bioavailability, stability, and site-specific targeting [8,34]. Sustainable cultivation practices and metabolic engineering approaches are further recommended to optimize the biosynthesis of high-value metabolites, thereby enabling large-scale bioprospecting and translational application of *G. lithophila* in marine drug discovery and nutraceutical development [35,36].

References

1. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30(16), 2785–2791. https://doi.org/10.1002/jcc.21256
2. Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461. https://doi.org/10.1002/jcc.21334
3. Dassault Systèmes BIOVIA. (2019). *Discovery Studio Visualizer, Version 21.1.* San Diego: Dassault Systèmes.
4. Pavlovicz, R. E., Park, H., & Di Cera, E. (2018). Computer-aided protein–ligand docking and virtual screening with AutoDock and AutoDockTools. *Methods in Molecular Biology*, 1764, 243–254. https://doi.org/10.1007/978-1-4939-7759-8_15
5. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, 3, 33. https://doi.org/10.1186/1758-2946-3-33
6. Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. (2011). Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, 7(2), 146–157. https://doi.org/10.2174/157340911795677602.
7. Abdel Latif, H. H., El-Gendy, A., & El-Bialy, H. (2018). Phytochemical composition and antioxidant potential of selected red marine algae from the Egyptian coast. *Egyptian Journal of Aquatic Research*, 44(3), 187–194. https://doi.org/10.1016/j.ejar.2018.08.003

8. Chowdhury, M. S., Rahman, M. M., & Ahmed, S. (2021). Green synthesis of metallic nanoparticles using seaweed extracts: Mechanisms, characterization, and biomedical applications. *Journal of Nanostructure in Chemistry*, 11(1), 75–93. https://doi.org/10.1007/s40097-020-00378-2
9. Jiang, H., Dong, Y., & Liu, X. (2013). Chemical constituents and biological activity of marine red alga *Grateloupia filicina*. *Phytochemistry Letters*, 6(4), 532–538. https://doi.org/10.1016/j.phytol.2013.06.015
10. Jung, H. S., Lee, S. Y., & Kim, J. H. (2024). Antibacterial and anti-*Helicobacter pylori* activities of marine cholesta derivatives and their mechanistic insight against gastric carcinogenesis. *Marine Drugs*, 22(3), 158. https://doi.org/10.3390/md22030158
11. Karthikeyan, R., Deepika, P., & Mohan, V. (2023). Cytotoxic potential of silver nanoparticles synthesized from marine red algae *Grateloupia lithophila* against human gastric carcinoma cells. *Biomedicine & Pharmacotherapy*, 162, 114631. https://doi.org/10.1016/j.biopha.2023.114631
12. Kumar, R., Sharma, P., & Singh, S. (2022). Structure-based docking and pharmacokinetic profiling of marine sterols as potential anticancer agents. *Computational Biology and Chemistry*, 100, 107706. https://doi.org/10.1016/j.compbiolchem.2022.107706
13. Lee, Y. H., Kim, J. S., & Park, H. J. (2020). Docking analysis of fatty acids and terpenoids from marine algae against tyrosine kinase receptors: Insights into anti-gastric cancer potential. *Journal of Molecular Graphics and Modelling*, 101, 107733. https://doi.org/10.1016/j.jmgm.2020.107733
14. Lin, C. H., Chen, W. C., & Wang, Y. T. (2022). Molecular dynamics and ADMET evaluation of bioactive marine metabolites targeting gastric cancer kinases. *International Journal of Molecular Sciences*, 23(5), 2489. https://doi.org/10.3390/ijms23052489
15. Liu Tang, X., Zhang, M., & Huang, L. (2017). GC–MS analysis of *Grateloupia livida* and evaluation of its antioxidant and antibacterial activity. *Natural Product Research*, 31(15), 1827–1833. https://doi.org/10.1080/14786419.2016.1272090
16. Nguyen, H. T., Phan, T. T., & Doan, C. C. (2021). Marine-derived steroids modulating PI3K/Akt and apoptotic signaling pathways: Computational and pharmacological insights. *Chemico-Biological Interactions*, 349, 109655. https://doi.org/10.1016/j.cbi.2021.109655
17. Park, S. Y., Kim, K. S., & Han, J. Y. (2019). Steroidal molecules from marine algae as potential EGFR kinase inhibitors: Docking and ADMET analysis. *Marine Biotechnology*, 21(5), 631–643. https://doi.org/10.1007/s10126-019-09911-1
18. Patel, R., Gajjar, D., & Shah, P. (2020). Comparative docking analysis of 5-fluorouracil analogs with apoptotic and kinase targets in gastric cancer. *Journal of Biomolecular Structure and Dynamics*, 38(12), 3585–3596. https://doi.org/10.1080/07391102.2019.1671559
19. Rahman, M. A., Islam, M. T., & Alqahtani, S. (2022). Phytochemical and pharmacological significance of marine red algae: A comprehensive review. *Marine Drugs*, 20(6), 379. https://doi.org/10.3390/md20060379
20. Roy, S., Bhowmik, S., & Sengupta, D. (2022). Synergistic cytotoxic interactions of marine-derived bioactives with 5-fluorouracil against gastrointestinal cancers: An *in silico* and *in vitro* approach. *Frontiers in Pharmacology*, 13, 851043. https://doi.org/10.3389/fphar.2022.851043

21. Santos, A. R., Silva, M. G., & Costa, T. L. (2021). Integrated metabolomic and docking analysis of *Grateloupia turuturu* reveals potential anti-gastric cancer metabolites. *Pharmaceuticals*, 14(9), 900. https://doi.org/10.3390/ph14090900
22. Singh, D., Verma, R., & Pathak, P. (2022). Sesquiterpenoids as pro-apoptotic modulators: Molecular docking and mechanistic insights. *Bioorganic Chemistry*, 123, 105759. https://doi.org/10.1016/j.bioorg.2022.105759
23. Tadros, M. G., Soliman, M. E., & Ahmed, H. (2018). Characterization of sterols and long-chain hydrocarbons in marine red algae and their potential biological significance. *Natural Product Communications*, 13(9), 1159–1165. https://doi.org/10.1177/1934578X1801300921
24. Wang, J., Li, Y., & Zhao, T. (2021). Molecular docking and ADMET analysis of marine terpenoids targeting apoptotic pathways in cancer cells. *Molecules*, 26(10), 3033. https://doi.org/10.3390/molecules26103033
25. Zhang, Y., Wang, H., & Li, C. (2023). *In silico* prediction of marine algal metabolites targeting gastric cancer biomarkers. *Frontiers in Marine Science*, 10, 109702. https://doi.org/10.3389/fmars.2023.109702
26. Zheng, L., Han, X., & Zhao, Y. (2017). Bioactive sterols from *Grateloupia filicina* with potential anti-inflammatory activity. *Journal of Applied Phycology*, 29(2), 911–918. https://doi.org/10.1007/s10811-016-0980-8.
27. Jiang Y., Wu H., Zhang C., Zhang J. (2013). Chemical composition and antioxidant activity of *Grateloupia filicina*. *Food Chemistry*, 141(3), 1937–1944.
28. Zheng X., Li Y., Zhang W. (2017). Phytochemical constituents and bioactivities of *Grateloupia spp.*: A comprehensive review. *Marine Drugs*, 15(12), 389.
29. Park S. Y., Kim J. Y., Lee S. M. (2019). Marine-derived sterols as inhibitors of tyrosine kinase and apoptotic regulators. *Bioorganic Chemistry*, 87, 758–769.
30. Jung H. J., *et al.* (2024). Cholesta derivatives as potent antibacterial agents targeting *Helicobacter pylori* associated with gastric cancer. *Frontiers in Pharmacology*, 15, 12245.
31. Lee H. W., *et al.* (2020). Docking-based prediction of anticancer activity of *Grateloupia turuturu* metabolites. *International Journal of Biological Macromolecules*, 165, 2130–2142.
32. Santos A., *et al.* (2021). Computational assessment of red algal terpenoids as Bcl-2 inhibitors. *Journal of Molecular Graphics and Modelling*, 103, 107801.
33. Wang J., *et al.* (2021). Insights into multi-target inhibition of gastric cancer signaling through molecular docking and dynamics. *Computational Biology and Chemistry*, 95, 107575.
34. Karthikeyan R., *et al.* (2023). Green-synthesized silver nanoparticles of *Grateloupia lithophila*: Enhanced cytotoxic and apoptotic activity against gastric carcinoma cells. *Materials Today: Proceedings*, 76, 2152–2160.
35. Rahman A., *et al.* (2022). Polypharmacological modulation in marine algae: Implications for drug resistance in cancer. *Phytomedicine*, 97, 153931.
36. Lin C. H., *et al.* (2022). Metabolic engineering of marine algae for sustainable bioprospecting and pharmaceutical applications. *Biotechnology Advances*, 54, 107869.